NWRI Contribution No. 89-133

# A PROCEDURE FOR THE DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS IN FISH.

by

J.P. Sherry[1] and H. Tse[2]

[1]Research and Applications Branch, National Water Research Institute, CCIW, 867 Lakeshore Rd., Burlington, Ontario L7R-4A6.
[2]National Water Quality Laboratory, CCIW, 867 Lakeshore Rd., Burlington, Ontario L7R-4A6.

## MANAGEMENT PERSPECTIVE

The determination of polychlorinated dibenzo-p-dioxins (PCDDs) in fish is a time consuming and laborious process. Method developments that improve analytical efficiency without compromising performance will benefit both routine and research programs. With that objective, some potential improvements to a previously used method for the analysis of PCDDs were investigated. A neutral solid phase extraction technique yielded equivalent results to a laborious and time consuming liquid phase acidic extraction technique. A multi-layer clean-up column, which replaces three separate liquid phase clean-up steps, was developed and evaluated with satisfactory results. The improved method was evaluated using a variety of fish samples. The enhanced efficiency and effectiveness of the described method will benefit the Directorate's PCDD surveillance and monitoring programs.

Dr. J. Lawrence
Director
Research Applications Branch

## PERSPECTIVE-GESTION

Le dosage des polychlorodibenzo-p-dioxines (PCDD) dans le poisson demande beaucoup de temps et beaucoup d'effort. Les améliorations qui permettront d'accroître l'efficacité des méthodes d'analyse sans pour autant en diminuer la performance seront avantageuses tant dans les programmes d'analyse de routine que dans les programmes de recherche. C'est avec cet objectif en tête que nous avons examiné certaines améliorations susceptibles d'être appliquées à une méthode qui servait auparavant au dosage des PCDD. Une technique d'extraction en phase solide neutre a donné des résultats équivalents à ceux obtenus avec une technique d'extraction en phase liquide acide qui était à la fois longue et laborieuse. Nous avons mis au point une colonne de purification à couches multiples qui permet de remplacer trois étapes distinctes de purification en phase liquide; l'évaluation de cette colonne a révélé qu'elle donnait des résultats satisfaisants. La méthode améliorée a été évaluée avec différents échantillons de poisson. L'efficacité accrue de la méthode décrite sera utilisée avantageusement dans le cadre des programmes de surveillance et de contrôle des PCDD mis en oeuvre dans la Direction générale.

M. J. Lawrence

Directeur

Direction de la recherche et des applications

## ABSTRACT

Some approaches designed to improve the efficiency of a method for the determination of polychlorinated dibenzo-p-dioxins in fish, without compromising performance, were explored. A solid phase extraction method was compared with aqueous phase acidic extraction: $^{13}$C-PCDD recoveries were comparable (>95%) for both methods. An effective multi-layer clean-up column was less time consuming than previously used liquid phase acid/base treatments. Using the modified method, $^{13}$C-$T_4$CDD recoveries were acceptable at low (10ppt), medium (20ppt), and high (100ppt) spiking levels; recoveries of $^{13}$C-$P_5$CDD through $^{13}$C-$O_8$CDD, which were determined at higher levels (2x for $P_5$CDD - $P_7$CDD; 3x for $O_8$CDD), were also satisfactory. The modified method was evaluated using a variety of fish samples; the results indicated acceptable performance: $^{13}$C-$T_4$CDD recoveries were $\geq$ 75%.

Nous avons étudié certaines techniques conçues dans le but d'améliorer l'efficacité, mais sans diminuer la performance, d'une méthode de dosage des polychlorodibenzo-p-dioxines dans le poisson. Nous avons comparé une méthode d'extraction en phase solide à une méthode d'extraction en phase aqueuse acide; dans les deux cas, les taux de récupération de $^{13}$C-PCDD étaient comparables (> 95 %). La purification sur une colonne efficace à couches multiples était plus rapide que la méthode par traitement acide/base en phase liquide qui était employée auparavant. Avec la méthode modifiée, les taux de récupération de $^{13}$C-PCDD étaient acceptables à des concentrations de dopage faibles (10 ppt*), moyennes (20 ppt*) et élevées (100 ppt*); les taux de récupération des congénères $^{13}$C-$P_5$CDD à $^{13}$C-$O_8$CDD, qui ont été déterminés à des concentrations de dopage plus élevées (2x pour les congénères $P_5$CDD à $P_7$CDD et 3x pour les congénères $O_8$CDD), étaient également satisfaisants. La méthode modifiée a été évaluée avec différents échantillons de poisson; les résultats obtenus révèlent que la performance est acceptable, les taux de récupération du $^{13}$C-$T_4$CDD étant ≥75 %.

---

*ppt = parties par billion ($10^{12}$)

## 1.0 INTRODUCTION

The determination of polychlorinated dibenzo-p-dioxins (PCDDs) in environmental samples is costly and time consuming. The identification, in recent years, of additional environmental inputs, such as pulp and paper mill effluents (Swanson et al. 1988; Clement et al., 1989), and the reported contamination of some food and consumer products with PCDDs (NRCC, 1981; Firestone et al., 1986; Beck et al., 1987, 1989; Ryan et al., 1988;) has led to a dramatic increase in the number and variety of samples requiring dioxin analysis. Moreover, the contamination from known sources must be quantified and monitored as the quest for further sources and problem areas continues.

Screening techniques, such as immunoassays, have the potential to reduce analytical overloads. However, a previous study (Sherry et al., 1989) indicated that considerable sample clean-up was required before fish extracts could be effectively analyzed by radioimmunoassay (RIA). The extraction and clean-up method (Afghan et al., 1987) used in that study was effective but unwieldy. With the objective of decreasing sample preparation time, neutral solid-phase extraction was compared with the previously used acidic extraction technique. A multi-layer clean-up column for the single step removal of several interference types was compared with liquid phase acid/base treatments. The resulting method was evaluated using a variety of fish samples.

## 2.0 MATERIALS AND METHODS

### 2.1 Fish Matrices

A Lake Trout (Salvelinus namaycush) (2.8 Kg, 20% lipid), taken from a polluted section of the eastern basin of L. Ontario, was used in the main experiments. Carp (Cyprinus carpio Linnaeus-), and Walleye (Stizostedion vitreum) were also used.

### 2.2 Spiking and Extraction Procedures

A portion of spiking solution, containing a mixture of surrogates ($^{13}$C-2,3,7,8-T4CDD; $^{13}$C-1,2,3,7,8-$P_5$CDD; $^{13}$C-1,2,3,4,-7,8-$H_6$CDD; $^{13}$C-1,2,3,4,6,7,8-$H_7$CDD; $^{13}$C-$O_8$CDD) in toluene, was spread over a shallow depression on the surface of the whole fish homogenate, and allowed to soak in for 1 h.

#### 2.2.1 Solid Phase (neutral; selected procedure).

Fish homogenate (10 or 25 g) was thoroughly ground in anhydrous $Na_2SO_4$ (200 g); the resulting mixture was transferred to a glass column (3 cm i.d. * 50 cm). The column was eluted with 800 mL of dichloromethane (DCM):hexane 1:1.

#### 2.2.2 Liquid Phase (acidic).

Fish tissue (10 g) was digested in a 500 mL Erlenmeyer flask, using 200 mL of 6 M HCl in toluene (1:1); the mixture was vigorously agitated using a wrist action shaker (12 h). Organic and aqueous phases were separated by centrifugation,

and the aqueous phase was re-extracted twice with toluene (Afghan et al., 1987).

## 2.3 Bulk Lipid Removal

After solvent removal, the extract was re-suspended (0.2 g/mL) in DCM:hexane (1:1) and clarified by filtration (5.0 um). The extract was chromatographed on Bio Beads S-X3 (60 g in a 2.5 cm * 60 cm column) using a GPC Autoprep unit (ABC Laboratories Inc.). The column was eluted (DCM:hexane (1:1)) at a flow rate of 5 mL/min.; the first 150 mL was discarded; PCDDs were eluted in the second 150 mL.

## 2.4 Additional Clean-up Steps

### 2.4.1 Liquid Phase Acid/Base Treatments.

The eluent from the GPC step was treated with base (0.05M $Na_3PO_4$) and acid (10N $H_2SO_4$) as described by Afghan et al., 1987.

### 2.4.2 Multi-layer Column (selected procedure).

Acidic silica 40% (w/w) was prepared by the stepwise addition of concentrated $H_2SO_4$ to activated silica gel. Similarly, basic silica 30% (w/w) was prepared using 1.5 M KOH, and $AgNO_3$ treated silica (10% w/w) was prepared using a solution of $AgNO_3$. The eluent from the GPC step was applied to the column

(Fig. 1) in 5 mL of 1% (v/v) toluene in hexane: toluene was included to minimize losses of $O_8CDD$. The column was eluted with 150 mL of 1% toluene in hexane.

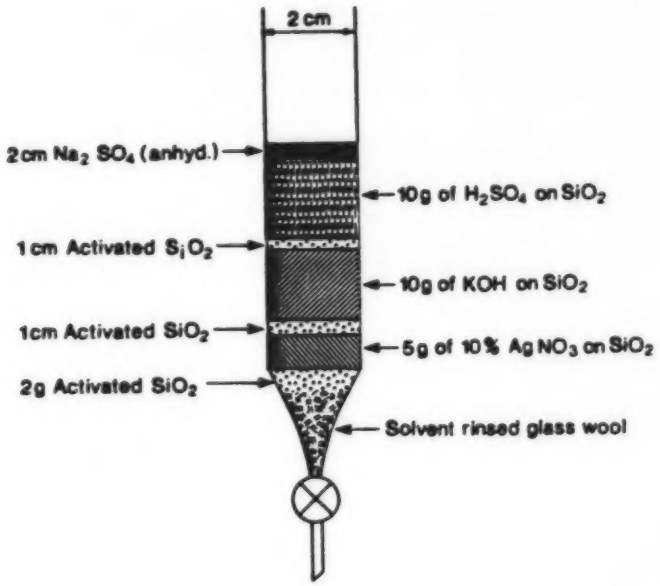


Fig. 1 Multi-layer clean-up column

2.4.3 Alumina and Carbon Fibre Chromatography.

Deactivated (1% w/w) basic alumina (Fisher) was packed (30 g) in a 2.5 cm i.d. glass column. Extract was applied to the column in 1% DCM in hexane; the column was eluted with 200 mL of the same solvent, and then with 150 mL of 50 % DCM in hexane. The latter fraction was transferred to 1 mL of DCM:cyclohexane (1:1), and chromatographed on activated carbon fibre

(Amoco PX 21 on shredded glass fibres (Afghan et al., 1987). PCDDs were recovered from the carbon fibre column by elution with toluene.

## 2.5 Quantitative Analysis (GC/MSD):

The enriched extract was re-suspended in toluene (25 uL) containing $^{13}$C-1,2,3,4-$T_4$CDD (50 pg/uL), and chromatographed (1 uL) on a 30 m HP-Ultra-2 capillary column using a Hewlett Packard gas chromatograph (GC) (HP5990A). The GC was coupled to a HP 5970 mass selective detector (MSD) (electron impact mode at 70 eV) operated in single ion monitoring mode. Quantification was based on the most intense ion in the molecular clusters; confirmation was based on the presence of at least one other cluster ion; the M - COCl ion was used to confirm native congeners. All ions were required to have the correct retention time, an acceptable peak shape, and the correct mass ratio. The instrument calibration standard, which contained the surrogates, and the corresponding unlabelled PCDD/PCDF isomers, was adjusted to match the samples' surrogate levels, and analyzed before and after every 3 samples. Native isomers and homologues were automatically corrected for the recovery of the corresponding $^{13}$C-PCDD surrogate, and were quantified using the relative response of the corresponding surrogate and unlabelled congener in the calibration standard.

## 3.0 RESULTS

### 3.1 Preliminary Evaluation:

The initial experiment was a replicated (n=3) comparison of liquid phase acidic extraction (Methods #1 - #4; (Table 1)) with solid phase neutral extraction (Methods #5 - #8; (Table 1)), using lake trout samples (10 g). As outlined in Table 1, an evaluation of several modifications to the reference clean-up procedure (Method #1) was incorporated into the experiment. The vortex (Method #2) and shake (Method #1) extraction techniques yielded similar surrogate recoveries (Table 2). Because of its gentler nature, vortex extraction reduces the risk of flask breakage or extract spills. The acidic (Methods #1 and #2) and neutral (Method #5) extraction techniques yielded comparable surrogate recoveries, when combined with liquid phase acid/base clean-up (Table 2). The limited data (n=3) indicates that acidic extraction was somewhat more precise than neutral extraction for the $^{13}C$-$P_5CDD$ - $^{13}C$-$H_7CDD$ congeners; however, the methods were equally precise for $^{13}C$-$T_4CDD$, and the CV% for $^{13}C$-$O_8CDD$ was apparently lower for the neutral extraction method.

Substitution of the solid phase chromatographic clean-up (SPC) step for the liquid phase acid/base clean-up (LPC) steps caused no apparent deterioration in surrogate recoveries, with either the acidic (Methods #1 (LPC) and #3 (SPC)) or neutral

(Methods #5 (LPC) and #8 (SPC)) extraction procedures (Table 2). With the exception of the $^{13}C$-$T_4CDD$ data for Method #8, solid phase clean-up was at least comparable in precision to liquid phase clean-up. Further experiments (Tables 3-5) demonstrated that the $^{13}C$-$T_4CDD$ data for Method #8 (Table 2) were atypical.

Elimination of the alumina chromatography step permitted gross interferences to pass through the clean-up, with the consequent loss of the $^{13}C$-$T_4CDD$ data (Table 2). Elimination of the acid/base clean-up steps (Method #6) was associated with erratic surrogate recoveries and imprecision (Table 2). Based on the foregoing data, Method #8 was selected for further evaluation.

**TABLE 1. Description of Evaluated Methods**

| | Method | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 6NHCl shake ex. | + | — | + | + | — | — | — | — |
| 6NHCl vortex ex. | — | + | — | — | — | — | — | — |
| Neutral column ex. | — | — | — | — | + | + | + | + |
| GPC | + | + | + | + | + | + | + | + |
| $Na_3PO_4$ wash | + | + | — | — | + | — | — | — |
| $H_2SO_4$ wash | + | + | — | — | + | — | — | — |
| Multi-layer column | — | — | + | + | — | — | + | + |
| Basic alumina | + | + | + | — | + | + | — | + |
| Carbon fibre | + | + | + | + | + | + | + | + |

TABLE 2. SURROGATE RECOVERIES USING A VARIETY OF METHODS

| 13C12 CONGENER | LEVEL (pg/g) | SAMPLE PREPARATION METHOD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| T4CDD | 125 | 94[1] (12)[2] | 98 (1) | 117 (13) | (3) | 89 (12) | 84 (50) | (3) | 150 (21) |
| P5CDD | 250 | 92 (11) | 106 (3) | 98 (6) | 130 (15) | 100 (17) | 95 (25) | 94 (14) | 105 (10) |
| H6CDD | 250 | 106 (11) | 109 (1) | 104 (7) | 123 (16) | 105 (19) | 107 (6) | 98 (8) | 111 (5) |
| H7CDD | 250 | 116 (6) | 105 (7) | 106 (5) | 126 (13) | 109 (20) | 94 (34) | 99 (6) | 115 (5) |
| 08CDD | 375 | 115 (33) | 124 (18) | 99 (13) | 119 (13) | 123 (17) | 80 (53) | 104 (21) | 114 (10) |

(1) Mean surrogate recovery (%) (n=3)
(2) CV% = Sx/$\bar{X}$.100
(3) Gross interference present

### 3.2 Method #8's Performance Using 3 Spike Levels:

The sample size was increased to 25 g for the following experiments. The data from the 100 ppt spike experiment (Table 3) indicated that both recoveries (98% - 105%) and precision (9% - 12%) were acceptable for each surrogate.

Recoveries (89% - 100%) and precision (6% - 17%) were also acceptable for each surrogate in the 20 ppt spike experiment (Table 4). Apart from losses of $^{13}C\text{-}O_8CDD$ in samples 1 and 2, no obvious deterioration in method performance was apparent. Native PCDD/PCDF congeners were also determined with acceptable precision (11% - 17%). The surrogate recoveries were also acceptable in the 10 ppt experiment (Table 5); although, in this case, decreased

$^{13}C$-$P_5CDD$ - $^{13}C$-$H_7CDD$ congener recoveries, without associated loss of precision, were observed. The data for the native congeners were acceptably precise (7% - 14%). The levels of native congeners and homologues were similar to those obtained using Method #1 (n=3): 2,3,7,8-TCDD = 34 pg/g; 2,3,7,8-TCDF = 37 pg/g; $P_5CDD$ = 8 pg/g; $H_6CDF$ = 10 pg/g.

TABLE 3. METHOD PERFORMANCE USING HIGH LEVEL SURROGATE SPIKE

| 13C12 CONGENER | LEVEL (pg/g) | REPLICATE 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | $\bar{X}$(1) | CV%(2) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T4CDD | 100 | 94 | 99 | 114 | 82 | 95 | 107 | 90 | 100 | 98 | 10 |
| P5CDD | 200 | 102 | 92 | 116 | 86 | 101 | 110 | 93 | 106 | 101 | 10 |
| H6CDD | 200 | 102 | 90 | 118 | 91 | 107 | 108 | 97 | 101 | 102 | 9 |
| H7CDD | 200 | 109 | 94 | 129 | 97 | 112 | 112 | 94 | 95 | 105 | 12 |
| 08CDD | 300 | 99 | 88 | 112 | 98 | 116 | 108 | 93 | 80 | 99 | 12 |

(1) Mean surrogate recovery (%)
(2) CV% = $Sx/\bar{X}.100$

TABLE 4. METHOD PERFORMANCE USING INTERMEDIATE LEVEL SURROGATE SPIKE

| CONGENER | LEVEL (pg/g) | REPLICATE 1 | 2 | 3 | 4 | 5 | 6 | 7 | $\bar{X}$(1) | CV%(2) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | RECOVERY (%) | | | | | | | | |
| 13C12-T4CDD | 20 | 94 | 84 | 98 | 94 | 87 | 91 | 101 | 93 | 6 |
| 13C12-P5CDD | 40 | 78 | 95 | 83 | 100 | 83 | 87 | 98 | 89 | 10 |
| 13C12-H6CDD | 40 | 85 | 93 | 101 | 114 | 90 | 83 | 104 | 96 | 11 |
| 13C12-H7CDD | 40 | 116 | 92 | 108 | 102 | 97 | 82 | 103 | 100 | 11 |
| 13C12-08CDD | 60 | 80 | 80 | 99 | 116 | 121 | 89 | 111 | 99 | 17 |
| | | CORRECTED(3) CONCENTRATION (pg/g) | | | | | | | | |
| 2,3,7,8-TCDD | | 36 | 47 | 33 | 48 | 35 | 37 | 34 | 38 | 16 |
| 2,3,7,8-TCDF | | 37 | 53 | 39 | 41 | 38 | 34 | 34 | 39 | 17 |
| P5CDF(4) | | 7 | 9 | 9 | 9 | 11 | 8 | 9 | 9 | 13 |
| H6CDF(4) | | 8 | 11 | 9 | 11 | 11 | 11 | 10 | 10 | 11 |

(1) Mean surrogate recovery (%)
(2) CV% = $Sx/\bar{X}.100$
(3) Data corrected for surrogate losses
(4) Total homologues

TABLE 5. METHOD PERFORMANCE USING LOW LEVEL SURROGATE SPIKE

| CONGENER | LEVEL (pg/g) | REPLICATE 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | CV%[2] | $\bar{X}$[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RECOVERY (%) | | | | | | | | | |
| 13C12-T4CDD | 10 | 81 | 85 | 100 | 105 | 97 | 115 | 97 | 87 | 12 | 96 |
| 13C12-P5CDD | 20 | 81 | 86 | 98 | 91 | 83 | 79 | 81 | 87 | 7 | 86 |
| 13C12-H6CDD | 20 | 92 | 106 | 103 | 91 | 78 | 77 | 94 | 85 | 11 | 91 |
| 13C12-H7CDD | 20 | 104 | 97 | 92 | 84 | 80 | 81 | 105 | 87 | 11 | 91 |
| 13C12-08CDD | 30 | 107 | 115 | 117 | 94 | 115 | 93 | 102 | 100 | 9 | 105 |
| | | CORRECTED[3] CONCENTRATION (pg/g) | | | | | | | | | |
| 2,3,7,8-TCDD | | 34 | 34 | 32 | 29 | 35 | 25 | 31 | 31 | 10 | 31 |
| 2,3,7,8-TCDF | | 36 | 23 | 39 | 37 | 36 | 31 | 38 | 34 | 15 | 34 |
| P5CDF[4] | | 8 | 8 | 9 | 8 | 8 | 9 | 8 | 8 | 7 | 8 |
| H6CDF[4] | | 11 | 10 | 10 | 10 | 10 | 13 | 12 | 14 | 14 | 11 |

(1) Mean surrogate recovery (%)
(2) CV% = Sx/$\bar{X}$.100
(3) Data corrected for surrogate losses
(4) Total homologues

TABLE 6. METHOD PERFORMANCE USING A VARIETY OF FISH SAMPLES

| 13C12 CONGENER | LEVEL (pg/g) | FISH SAMPLE (LAB. #) CARP (JH1) | CARP (JH2) | L. TROUT (JH3) | CARP (JH4) | L. TROUT (JH5) | L. TROUT (JH6) | L. TROUT (JH7) | L. TROUT (JH8) | WALLEYE (JH9) | CARP (JH10) | $\bar{X}$[2] | CV%[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T4CDD | 50 | 99[1] | 94 | 86 | 94 | 75 | 109 | 113 | 118 | 95 | 127 | 101 | 16 |
| P5CDD | 100 | 78 | 78 | 93 | 90 | 73 | 80 | 78 | 90 | 87 | 94 | 84 | 9 |
| H6CDD | 100 | 67 | 79 | 89 | 102 | 81 | 63 | 56 | 86 | 80 | 121 | 80 | 24 |
| H7CDD | 100 | 78 | 100 | 89 | 89 | 80 | 72 | 74 | 89 | 82 | 90 | 85 | 1 |
| 08CDD | 200 | 67 | 109 | 129 | 83 | 96 | 78 | 101 | 88 | 82 | 103 | 94 | 19 |
| Lipid Content (%) | | 9.8 | 10.7 | 12 | 11 | 13.6 | 16.3 | 16.5 | 19.6 | 10 | 9 | | |

(1) Surrogate recovery (%)
(2) Mean surrogate recovery (%)
(3) CV% = Sx/$\bar{X}$.100

### 3.3 Method Performance Using a Variety of Fish Samples:

Method #8 was evaluated using 10 fish samples (25 g) (Table 6). Blockages of the GPC unit occurred during chromatography of JH1, JH6, JH7, and JH8; some extract losses occurred. High lipid extracts should probably not be allowed to remain in the sample loops longer than necessary, so as to reduce the possibility of line blockage. Despite this problem, surrogate recoveries were generally acceptable, and would probably improve with sample replication. In only one case (JH5) was the $^{13}C$-$T_4CDD$ recovery less than 80%. Some $^{13}C$-$O_8CDD$ recoveries were lower than the corresponding $^{13}C$-$T_4CDD$ values. Although recoveries tended to be lower for the $^{13}C$-$P_5CDD$ - $^{13}C$-$H_7CDD$ surrogates, in no case was an uncorrectable loss incurred.

## 4.0 DISCUSSION

Method #1 (Afghan et al., 1987), was modified for the present study by increasing the extraction acid's strength from 1 - 6 M, and incorporating an automated GPC step. GPC automation boosted sample throughput from 1.5 samples to 8 samples per day. Emulsion problems associated with Method #1's liquid phase steps do not, in our experience, facilitate the analysis of large (> 10g) fish samples. Solid phase procedures, are free of such problems. The presented data, while of a preliminary nature, verify that solid phase extraction and clean-up can improve ana-

lytical efficiency and effectiveness.

Solid phase extraction, which has been used by Norstrom (1986) and Smith et al. (1984), among others, is efficient. It facilitates the simultaneous and efficient extraction of multiple samples: a single operator can extract 16 samples a day. Whereas, liquid phase extraction permits the simultaneous extraction of about 6 samples by a lone operator. Furthermore, solid phase extraction has fewer equipment and glassware requirements.

The elimination of either the acid/base treatments or the alumina chromatography step compromised analytical performance. Elimination of the former was associated with erratic surrogate recoveries, possibly caused by interference of residual lipids or biogenic molecules with the alumina or carbon fibre chromatography. Such inconsistent performance would probably worsen with larger samples. Similar considerations apply to the elimination of the alumina chromatography step; in this case, the gross interferences that co-chromatographed with $^{13}$C-2,3,7,8-TCDD were probably PCBs.

The multi-layer clean-up column, which is based on the "DOW" clean-up strategy (Lamparski and Nestrick, 1980) offers several practical advantages. Large samples can be processed without encountering emulsion associated problems. Multiple samples can be processed simultaneously, 20 per day being a rea-

sonable number for a single operator. In contrast, it takes a single operator about 2 days to process 8 samples using liquid phase acid/base treatments. Material costs are also lower for the solid phase method.

The correction of native PCDDs/PCDFs for surrogate losses did not consistently improve analytical precision, although it tended to increase analyte estimates in the low and intermediate spike experiments. The failure to improve analytical precision for the PCDF parameters may result from the use of the corresponding PCDD surrogate to correct for method losses. However, the reason for the lack of improvement in the precision of the corrected 2,3,7,8-TCDD data is not clear. Correlation analysis failed to detect a consistent relationship between the surrogate and native 2,3,7,8-TCDD recoveries ($P>0.05$).

Further validation of Method #8's analytical precision, using fish matrices that contain a variety of congener profiles, is required: some of the fish listed in Table 6 would be suitable. Participation in round robin studies would enable conclusions to be drawn regarding the comparative performances of Method #8 and other methods, while also providing information on analytical accuracy. Method #8 should be readily adaptable for use with other matrices, although validation would be required in each case.

## 5.0 REFERENCES

Afghan, B.K., J. Carron, P.D. Goulden, J. Lawrence, D. Leger, F. Onuska, J. Sherry and R.J. Wilkinson (1987). Recent advances in ultratrace analysis of dioxins and related halogenated hydrocarbons. Can. J. Chem. 65: 1086-1097.

Beck, H., K. Eckart, W. Mathar, Ch.-S. Ruhl and R. Wittkowski (1987). Levels of PCDFs and PCDDs in samples of human origin and food in the Federal Republic of Germany. Chemosphere 16: 1977--1982.

Beck, H., A. Drob, K. Eckart, W. Mathar and R. Wittkowski (1989). PCDDs, PCDFs and related compounds in paper products. Chemosphere 19: 655-660.

Clement, R.E., S.A. Suter, E. Reiner, D. McCurvin and D. Hollinger (1989). Concentrations of chlorinated dibenzo-p-dioxins and dibenzofurans in effluents and centrifuged particulates from Ontario pulp and paper mills. Chemosphere 19: 649-654.

Firestone, D., R.A. Niemann, L.F. Schneider, J.R. Gridley and D.E. Brown (1986). In: Chlorinated Dioxins and Dibenzofurans in Perspective (C. Rappe, G. Chaudhary, and L. Keith, eds.), pp. 355-365. Lewis Publishers Inc., Michigan.

Lamparski, L.L. and T.J. Nestrick (1980). Determination of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxin isomers in particulate samples at parts per trillion levels. Anal. Chem. 52: 2045--2054.

Norstrom R.J., M. Simon and M.J. Mulvihill (1986). A gel-permeation/column chromatography clean-up method for the determination of CDDs in animal tissue. Intern. J. Environ. Anal. Chem. 23: 267--287.

NRCC, (1981). Polychlorinated dibenzo-p-dioxins: criteria for their effects on man and his environment. NRCC # 18574. National Research Council of Canada.

Ryan, J.J., L.G. Panopi and D.A. Lewis (1988). Bleaching of pulp and paper as a source of PCDDs and PCDFs in food. Presented at Dioxin '88: The 8th International Symposium on Chlorinated Dioxins and Related Compounds, Abs. # SOU 07, Aug. 21-26 Umea, Sweden.

Sherry J., J. ApSimon, L. Collier, R. Wilkinson, P Albro and B. Afghan (1989). The use of radioimmunoassay for the detection of polychlorinated dibenzo-p-dioxins in fish samples. Chemosphere 19: 255-261.

Smith, L.M., D.L. Stalling and J.L. Johnson (1984). Determination of part per trillion levels of polychlorinated dibenzofurans and

dioxins in environmental samples. Anal. Chem. 56: 1830-1842.

Swanson, S.E., C. Rappe, J. Malmstrom and K.P. Kringstad (1988). Emissions of PCDDs and PCDFs from the pulp industry. Chemosphere 17: 681-691.